Esp

Week 5

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Transformation of pSB1K3-T7-EB into BL21

Responsible

Shuangjia Xue, Oskar Ohman

Protocols used

- Transformation

Experimental Set Up

Amount of plasmid	5 μl
Amount of BL21 cells	50 μl

Plasmid extracted from two different liquid cultures was used. Cells were heat shocked for 15 seconds and the entirety of the cell suspension plated onto kanamcin agar plates.
This experiment was performed twice in this week without significant changes. The reason was that the protein which was expressed with cells from this transformation was frozen at -20°C which might affect functional testing. In order to produce fresh protein that could be tested immediately, this second transformation was conducted.

Results and Conclusions

No results at this stage but the next day many colonies were observed.

Discussion and Troubleshooting

Transformation of plasmids into BL21 worked fine as expected. In a next step, liquid cultures can be prepared for protein expression.

2 IPTG-induced Expression of pSB1K3-T7-EB and Sonication

Responsible

Sigrun Stulz

Protocols used

- Protein Expression - Sonication

Modifications to protocols

25 ml of liquid culture were prepared instead of the proposed 10 ml.

Experimental Set Up

Five colonies were picked from each of the two plates and inoculated in 25ml LB with 35μg/ml kanamycin. They were grown until they reached OD600 0.6
Subsequently they were induced with 1.0mM or 0.5mM for 3 or 4 hours. The samples were collected, spun down at 3000 rpm, 5 min, 4°C and the pellet was frozen.

Table 1: Protein Expression Samples

IPTG concentration	Incubation time
uninduced	4h
1mM	3h
1mM	4h
0.5mM	3h
0.5mM	4h

Sample Calculation

To achieve concentrations of 1mM and 0.5mM IPTG from 500mM stock concentration in 25ml

Dilution for 1.0mM:	500mM / 1mM = 500
Amount of stock solution added to 25 ml:	25ml / 500 = 0.05 ml = 50 μl
Dilution for 0.5mM:	500mM / 0.5mM = 1000
Amount of stock solution added to 25 ml:	25ml / 1000 = 0.025 ml = 25 μl

Results and Conclusions

No results at this stage. Cells will be lysed and SDS-Page performed to assess protein expression.

3 SDS-Page of EB

Responsible

Shuangjia Xue, Oscar Frisell

Protocols used

- Sonication - SDS-Page

Experimental Set Up

Cells were thawed, washed in 10ml PBS and resuspended in 5ml cell lysis buffer. They were sonicated and spun down at 4000 rpm, 4°C, 15minutes and supernatant and pellet were run separately in order to assess whether the protein was soluble or not.
Consecutively, SDS-Page gel was prepared according to protocol and the samples prepared in sample buffer and boiled before running them.

Results and Conclusions

Analysis of an SDS-Page in this case can be a bit trickier because Esp is originally transcribed as a pre-pro-protein which would have a theoretical size for EB is 30.24 kDa, based on protein size calculators such as the Molecular Weight Calculators at ExPASy but has experimentally been shown to have a size of 27 kDa. Additionally, in S.epidermidis, Esp is typically cleaved into an active form (theoretically of a size of 24kDa) but this is unlikely to happen spontaneously in E.coli. A weak band can be seen in the induced, insoluble samples but it is unclear whether it corresponds to the exact size of Esp. For the calculator, see web.expasy.org/$compute_pi$/, retrieved 22.09.2016

Table 2: SDS-Page

Well	Sample	Treatment
1	Ladder	
2	EB 1	soluble fraction induced
3	EB 1	soluble faction uninduced
4	EB 2	soluble fraction induced
5	EB 2	soluble fraction uninduced
6	Ladder	
7	EB 1	insoluble fraction induced
8	EB 1	insoluble fraction uninduced
9	EB 2	insoluble fraction induced
10	EB 2	insoluble fraction uninduced

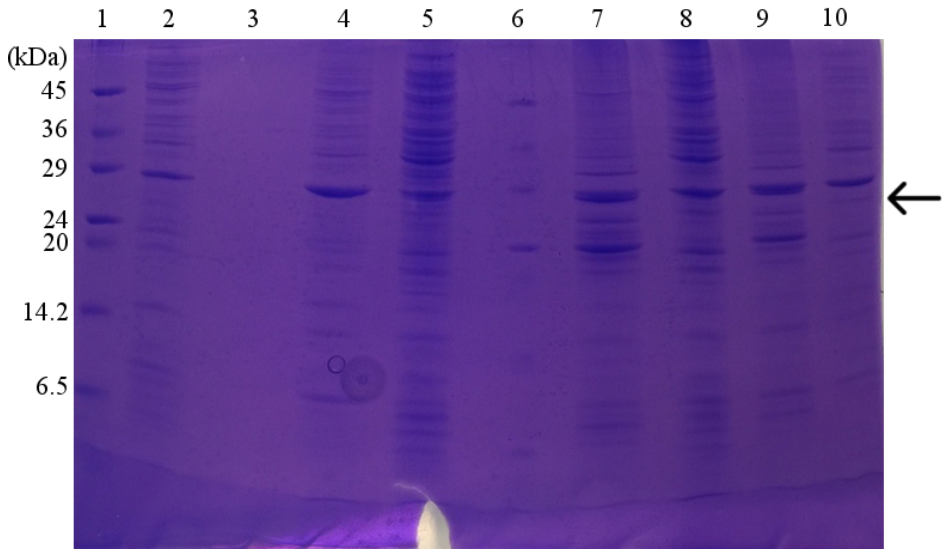

Figure 1: SDS-Page analyzing EB expression. The arrow indicates a band observed in the pellet of induced cells corresponding to the expected size of EB.

Discussion and Troubleshooting

Analyzing this SDS-Page gel is troublesome for a variety of reasons. First of all, our ladder did not separate well and instead of the expected 8 bands we could only observe 7. Based on previously run gels we were able to deduce that the biggest protein in the ladder (at a size of 66kDa) had probably not shown up. Additionally, no obvious, strong band in the correct size was seen and the Gel was quite crowded, making interpretation difficult.
In a next future, a similar gel could be run again, hoping for better separation of the ladder or with a prestained ladder where differentiating the bands would be easier due to their different colours. Ideally, a Western Blot could be performed but antibodies for Esp are not widely commercially available.

References

Sugimoto, S. et al. Cloning, expression and purification of extracellular serine protease Esp, a biofilm-degrading enzyme, from Staphylococcus epidermidis. J. Appl. Microbiol. 111, 14061415 (2011).

4 Overhang PCR of pSB1K3-T7-EB

Responsible

Bethel Tesfai Embaie, Reskandi Rudjito, Shuangjia Xue

Protocols used

- PCR, Q5 high fidelity Master Mix

Modifications to protocols

Three lysostaphin plasmids were run in parallel which is why the Master Mix is calculated for more samples than represented here.

Experimental Set Up

This experiment was performed in order to add a BamHI restriction site to the 3'end of EB, which will be needed to add the Linker-Tag sequence.
Both colonies from EB were run here with two different PCR programs, where the annealing temperature was either 52°C or 57°C to optimise primer binding. Primers were always used at a concentration of 10 μM.

Table 3: Experimental Set Up

Sample	**Component**
Overhang EB1	VF2_F + EB_R
Overhang EB2	VF2_F + EB_R
EB pos. control	VF2_F + VF2_R
neg. control	no DNA
EB plasmid	no PCR reaction

Table 4: PCR Programs

Step	**Temperature °C**	**Time**
Initial Denaturation	98	30s
	98	10s
Main Part: 30 cycle	52 or 57	30s
	72	20s
Final Extension	72	2min
Hold	4	infinite

Results and Conclusions

Table 5: PCR 52°C

Well	Sample	Description
1	Ladder	
7	Overhang EB1	multiple bands (unspecific)
8	Overhang EB2	multiple bands (unspecific)
9	EB pos. control	strong PCR product
10	EB plasmid	faint band at 2000-3000 bp
11	Negative control	smear

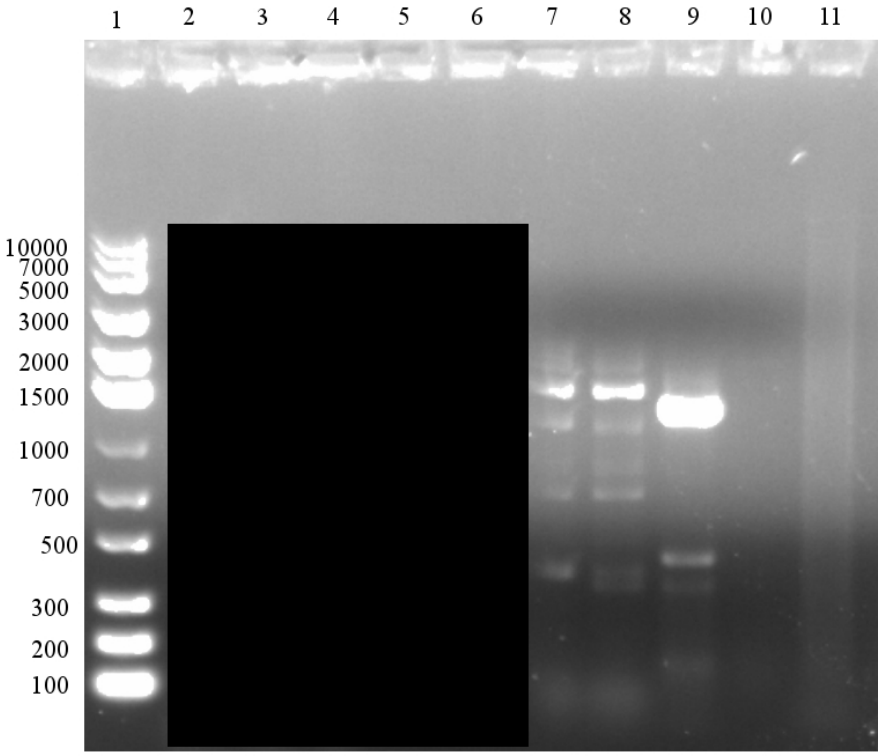

Figure 2: Analysis of overhang PCR with 52°C annealing temperature

Table 6: PCR 57°C

Well	Sample	Description
1	Ladder	
6	Overhang EB1	no product
7	Overhang EB2	no product
8	EB pos. control	strong PCR product
9	Negative Control	Faint band at 2000-3000 bp

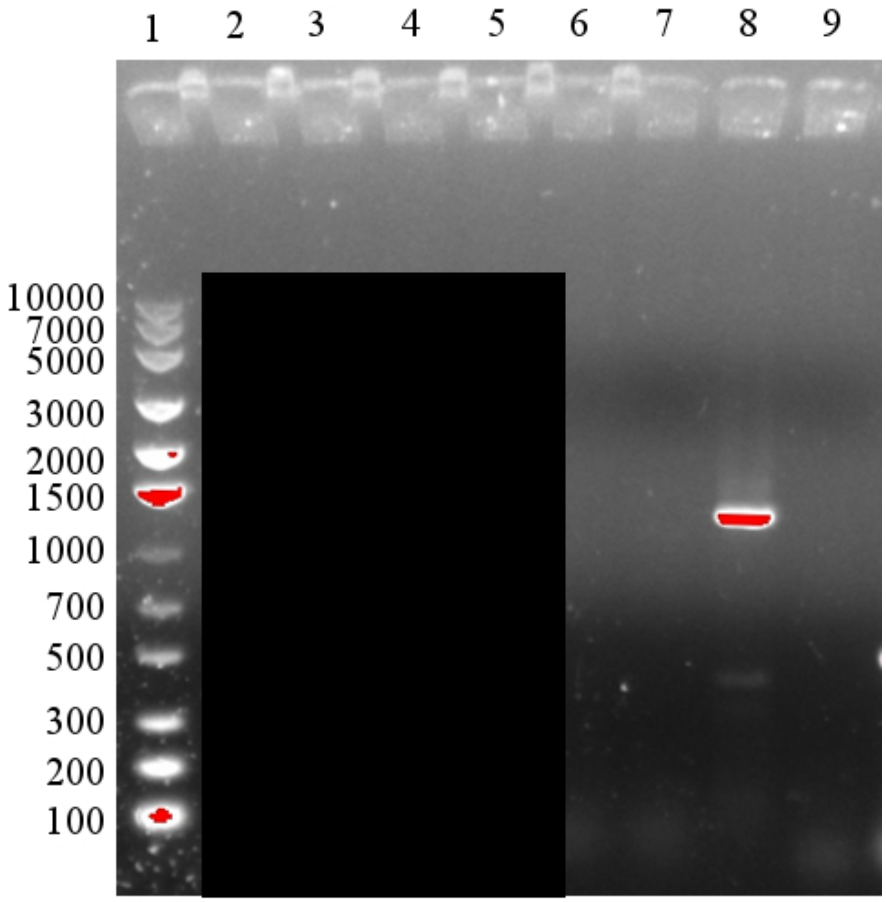

Figure 3: Analysis of overhang PCR with 57°C annealing temperature

Discussion and Troubleshooting

The PCR seems to have been largely unsuccessful with PCR products that were either unspecific (at 52°C annealing temperature) or simply not present (57°C). Alongside the annealing temperature, further components of the PCR such as primer and MgCl concentration can be optimised. Additionally, it could be beneficial to use Taq polymerase again, which has been used more extensively in this lab than the Q5 Master Mix.